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(57) Abstract

A fibrinogen based composition is described which is derived from bovine plasma through the use of polyethylene glycol ("PEG") and glycine precipitation techniques. The composition contains highly purified fibrinogen which can be activated and converted to fibrin by the addition of thrombin or by the action of endogenous thrombin in the case of small wound closures for purposes of hemostasis. Additionally the fibrinogen composition provides sufficient strength to be used as a surgical adhesive. A test kit is also provided for detecting allergy to bovine-derived fibrinogen.

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FIBRINOGEN BASED ADHESIVE

This application is a continuation-in-part of United States Patent Application Serial No. 652,332 filed on February 7, 1991.

The invention described herein relates to an adhesive composition which is useful for the surgical repair of tissue as well as for its hemostatic effects.

Additionally, the invention described herein encompasses a test kit for detecting potentially untoward allergic reactions which may be experienced by patients who receive the fibrinogen based adhesive.

BACKGROUND OF THE INVENTION

The invention described herein addresses fibrinogen-containing adhesives which utilize plasma as a fibrinogen source, the preferred plasma being bovine plasma. Hence, the transmission of blood-borne human diseases which are of concern whenever pooled human plasma is used may be avoided. Consequently, one objective of the present invention is to facilitate the use of fibrinogen-based adhesives without requiring the use of autologous or single donor fibrinogen to reduce the transmission of most blood-borne diseases.

Another objective of the invention described herein relates to the use of higher PEG's.

A further aspect of the present invention relates to an improved fibrinogen based adhesive suitable for effecting wound closures.

Yet another aspect of the invention described herein relates to a fibrinogen based adhesive to facilitate wound healing.

Other advantages to the methods of preparation and the fibrinogen based adhesive described herein will be apparent to those of

ordinary skill in th art from the teachings herein.

SUMMARY OF THE INVENTION

The present invention relates to a fibrinogen-based adhesive which contains fibrinogen precipitated from plasma with polyethylene glycol. The plasma is treated with PEG to form a precipitate. The precipitate is re-treated with polyethylene glycol to further purify the precipitate. The purified precipitate is then treated with glycine to form a glycine-purified precipitate. The glycine-purified precipitate is reconstituted and lyophilized to form a dry composition containing about 90 to about 98 percent fibrinogen, about 0.5 to about 2 to 3 percent fibrin, less than about one percent fibronectin and less than about one percent factor XIII (FXIII).

DETAILED DESCRIPTION

The present invention utilizes a fibrinogen-based adhesive prepared in the manner described below, and having the properties noted.

As used herein, the term "fibrinogen" is used in the conventional sense to refer to human or animal plasma protein of relatively high molecular weight that is converted to fibrin. As those of ordinary skill in this art will understand, this may occur through the action of thrombin, calcium and other coagulation factors.

Also referred to as factor I or Parenogen, fibrinogen is a glycoprotein belonging to the keratin-myosin group. Fibrinogen is synthesized and secreted by hepatic parenchymal cells, and is present in normal human plasma at levels of about 0.3 to 0.4 g/100 ml.

The fibrinogen molecule is believed to contain three peptide chains, alpha (A), beta (B)

and gamma (C), which are cr sslinked by disulfide bridges. The molecular weight is approximately 400,000 for the dimeric form.

The term "fibrin" is used herein to refer to the protein which is formed from fibrinogen. As those of ordinary skill will understand, this may occur through the action of thrombin, calcium and other coagulation factors. Fibrin typically refers to polymerized fibrin monomers, and may be present in at least two forms; "fibrin-i", which is insoluble fibrin, formed through the reaction of a fibrinogen-like plasma protein, ("FSP"), which in the presence of calcium, converts from a weakly bonded gel into a covalently bonded, insoluble clot. "Fibrin-s" refers to fibrin which is soluble in urea.

"Thrombin", as used herein, refers to a serine based protease which is derived from prothrombin, which reacts to form thrombin through the action of thromboplastin in the presence of calcium ions. Bovine thrombin contains two polypeptide chains designated chain A and chain B. Chain A contains 49 amino acid residues. It is bound via disulfide linkages to chain B, which contains about 265 amino acid residues as well as carbohydrate.

"Prothrombin" refers to the precursor compound of thrombin. Also known as factor II, prothrombin is a coagulation pro-enzyme having a molecular weight of 69,000 to 74,000. Prothrombin's fibrinogen-activating effect is vitamin-K dependent. It is converted to thrombin by the combined action of factor X_a, factor V and phospholipid in the presence of divalent calcium ions. It is believed to account for less than 0.2% of the total plasma protein, and is most stable within the range of pH 4 to 9.5. Prothrombin is precipitable at pH 4.2 to 4.5. The

dry material (dried from the frozen state) may show reduced activity after a few months. Drying prothrombin with an organic solvent typically destroys its activity.

In the normal coagulation process, injured tissue is initially contacted with blood, resulting in the activation of factor XII (also known as Hageman factor). Factor XII is believed to react with calcium, plasma thromboplastin antecedent (also known as factor XI or "PTA"), plasma thromboplastin component (also known as Christmas factor, factor IX and "PCT"), antihemophilic globulin (also known as factor VIII and "AHG"), thromboplastin (also known as factor III), labile factor (also known as factor V) and Stuart-Prower Factor (also known as factor X) to produce intrinsic or blood thromboplastin. This cascade typically occurs in about three to five minutes.

Extrinsic or tissue thromboplastin is formed rapidly (in less than about 12 seconds) in various tissues, e.g., the lung and brain, in the presence of factor V, factor VII (also known as Stabile Factor, proconvertin, serum prothrombin conversion accelerator and "SPCA"), and factor X.

In stage two of the normal clotting mechanism, thromboplastin catalyzes the conversion of prothrombin to thrombin in the presence of factors V, VII and X and calcium.

In stage three, thrombin rapidly converts fibrinogen into fibrin, which forms a fiber network. The network traps red blood cells, thus forming a blood clot.

In addition to the clotting factors described above, blood typically also contains natural clot inhibitors, such as antithrombin, heparin and antithromboplastin, each of which can interfere with the clotting mechanism cascade.

It is believed that the natural balance between the factors described above facilitates clotting at the appropriate time and site. This prevents unnecessary premature clotting, while meeting the needs of the organism when clotting is required, such as to prevent undesirable blood loss from a wound.

The invention described herein includes a process for producing a fibrinogen-based adhesive having high levels of purity and tensile strength after it has cured and a high level of reproducability in the extraction/purification process.

Typically a mammalian source of plasma, e.g., cow or pig, is bled, and the whole blood is treated using a plasmapheresis unit to obtain the plasma. Alternatively, other methods of obtaining plasma may be used. By selecting a nonhuman mammalian source for plasma, most blood-borne diseases which affect humans, e.g., hepatitis, etc., can be avoided. The selection of a particularly well-suited species for the production of plasma coupled with carefully controlled and monitored diet and living conditions, as well as routine screening for pathogenic contamination, further reduces the likelihood of transmission of blood borne diseases to humans.

The preferred source of plasma is the cow, since there is only a small degree of disparity between human fibrinogen and bovine fibrinogen.

The plasma is drawn into an anticoagulant solution, e.g, acid-citrate-dextrose solution ("ACD") or another suitable anticoagulant containing solution.

The plasma is treated with polyethylene glycol for a time period and at a concentration and temperature which are effective for precipitating

the fibrinogen which is contained in th plasma, without substantially denaturing the fibrinogen or precipitating a high concentration of non-fibrinogen plasma proteins, and without converting the fibrinogen to fibrin prematurely to any substantial degree which would render it inactive for use as an adhesive or hemostatic agent. Polyethylene glycol is used as the precipitating agent in the invention described herein for bovine fibrinogen, since it does not precipitate all of the non-fibrinogen plasma protein components, it is biocompatable and has a relatively consistent molecular weight when purchased from reliable commercial suppliers in the appropriate purity grade. The preferred PEG for use herein is PEG-8000. It exhibits biocompatability and enables one to achieve higher levels of purity (and fibrinogen yield) than other PEG preparations. PEG-8000 is commercially available in different purity grades, and the highest purity level, such as that produced by Sigma Chemical Corp., is the most preferred.

The plasma is treated with PEG using an effective amount thereof to precipitate the fibrinogen from the plasma. An effective concentration of PEG is about 50g/liter of plasma. This treatment is continued until a fibrinogen-containing precipitate is formed. This initial precipitation step typically takes about 10 to 30 minutes for precipitation to be complete.

The PEG precipitation step may be repeated two or three times at a temperature which does not adversely affect the precipitation reaction, and which does not substantially denature the fibrinogen. This precipitation step is most preferably conducted at about 5°C to about 23°C.

The PEG is typically added to the plasma in a buffered solution. One preferred buffer

utilizes a combination of monosodium phosphate, sodium chloride, sodium citrate and epsilon aminocaproic acid. Other buffer systems which are useful in this regard are TRIS, phosphate, borate and bicarbonate systems.

The PEG/buffer solution is added to the plasma gradually over about 15 minutes with constant stirring, until the fibrinogen precipitates.

The precipitate is collected by centrifugation at, e.g., about 4000 rpm, at a reduced temperature, e.g., about 5°C over about twenty minutes. The supernatant containing non-fibrinogen plasma proteins may then be decanted off and discarded. The precipitate is then purified with repeated PEG/buffer treatments until the desired purity level is attained. Preferably this precipitation reaction is conducted two times, with the shortest time possible between precipitation and redissolution of the precipitate in a buffer solution.

After the PEG purification is complete, the precipitate is redissolved in buffer, and the dissolved precipitate is treated with glycine in a concentration of about 1.5 to 2.5M. The preferred glycine concentration is about 2.1M. The glycine treatment is continued until the precipitate is produced. The glycine precipitation step produces a less flocculated precipitate than that seen with PEG-8000 precipitation, and is of higher purity. This precipitate is also resuspended in buffer solution as soon as possible after the precipitate is centrifuged and the supernatant is decanted off.

After completing this treatment with glycine, and redissolving the precipitate in buffer solution, the solution is lyophilized to form the fibrinogen containing powder.

Lyophilization may be conducted at a

cryogenically effective temperatur , e.g., at least as low as about -50°C to about -70°C, although liquid nitrogen temperatures, e.g., about -196°C, may be used. Pressure is reduced below ambient, and the shelves contained within the lyophilizer and preparation to be lyophilized are heated to about the eutectic point for fibrinogen, and the other components contained therein. This enhances the freeze drying effect of the lyophilization process and is useful for eliminating water from the preparation without substantially reducing the fibrinogen yield which is obtained. The reduced temperature and pressure, along with the temperature differential between the atmosphere and the shelf, can be maintained in this fashion for an appropriate time period, e.g., from about 4 hours to as long as about 1 week to fully lyophilize the product.

Near the end of the lyophilization process, when a change in the preparation becomes visually apparent, ambient air is permitted to enter the lyophilizer. This also enhances the yield achieved and reduces any unwanted protein denaturation or premature conversion of fibrinogen to fibrin.

The fractionation procedure described above produces a composition containing at least about 90 to about 98 percent fibrinogen with a low level of conversion to fibrin, and very low levels of precipitated non-fibrinogen plasma proteins. Other components include, e.g., about 0.5 to about 2 to 3 percent fibrin, less than about 1 percent fibronectin and less than about 1 percent factor XIII.

It is believed that the buffer system is important in that it minimizes the premature, unwanted conversion of fibrinogen to fibrin, minimizes the denaturation of fibrinogen and

facilitates high yields. The most preferred buffer system for us herein is as follows:

Monosodium Phosphate	0.0170M
Sodium	0.141M
Sodium Citrate	0.009M
Epsilon Aminocaproic Acid	0.1M

This buffer system is compatable with polyethylene glycol and glycine, and is useful for the PEG 8000 and glycine precipitation and intermediate reconstitution steps as well as for the final reconstitution prior to lyophilization.

The osmolarity of the buffer system noted above is about 0.267 and the pH is about 7.3. The low concentration of citrate is felt to enhance the purification of fibrinogen while minimizing the unnecessary premature conversion of fibrinogen to fibrin. Most other buffer systems with about a 0.009M citrate level or other inhibiting substances or buffers of varying concentrations facilitate similar results.

The time intervals between collection and re-dissolution of the precipitate, as well as the time interval between the final glycine-precipitation step and lyophilization should be kept as short as possible to minimize any undesirable denaturation of fibrinogen or premature conversion to fibrin. The redissolution steps should typically be initiated within about 10-15 minutes.

After lyophilization, the powder is packaged under sterile conditions in pharmaceutically acceptable containers, such as packets, vials or bottles, depending upon the intended use. These packages may be sized to accommodate small or large quantities of adhesive, depending upon the expected needs of the physician, and should be light resistant and stored at

refrigerator temperatures, e.g., about 5°C prior to use.

Numerous pharmaceutical inert or active additives, compatible with the fibrinogen and the buffer system described above may be included in the formulation, such as diluents, preservatives, dispersants and the like.

Preservatives, when used, are typically added to the fibrinogen powder after lyophilization to minimize untoward reactions with the fibrinogen, but may be added prior to lyophilization if appropriate. Representative examples of preservatives include thimerosal, antibiotics, BHA, BHT, sorbic acid, sodium metabisulfite and the like. These compounds may be added in amounts which are preservative effective without substantially denaturing or inactivating the fibrinogen, and without adversely affecting the buffer system. Typical concentrations range from about 0.0001 to about 0.01 percent, based upon the weight of the lyophilized powder prior to reconstitution.

When the adhesive is to be applied to an incision after surgery as a means of wound closure, the powdered fibrinogen may be dissolved with diluent and gently shaken. The preferred diluent is sterile distilled water, but other diluents can be used as well. A sufficient amount of diluent is added to provide a solution containing about 1 to 40 mgs. of fibrinogen per ml. The fibrinogen is dissolved slowly with gentle shaking since vigorous agitation can cause denaturation of the fibrinogen or the premature conversion of fibrinogen to fibrin, thus rendering it less effective. The preparation, when dissolved in water, appears as a white insoluble material; as it becomes hydrat d, the solution becomes translucent and then finally clear over several minutes.

The fibrinogen solution is activated by combining it with thrombin. Numerous commercial thrombin preparations are available which may be used to activate the fibrinogen composition, converting the fibrinogen to fibrin. Typical thrombin preparations contain about 1 unit to 1000 units per milliliter.

Different ratios of fibrinogen to thrombin may be used for different applications, and the tensile strength of the adhesive after setting may vary slightly with the concentration and quantity of thrombin added to the fibrinogen solution. To form the adhesive preparation and activate the fibrinogen, the appropriate thrombin solution is combined with the appropriate fibrinogen solution, and the liquid combination is applied to the operative site for wound closing or is applied to a vessel for its hemostatic effect. In general, set times range from about 20 sec. to as long as about one hour. The set time can be shortened or lengthened to some extent by changing the concentration and amount of thrombin which is added to the fibrinogen.

Alternatively the fibrinogen and thrombin solutions may be applied to the tissue site separately.

The lyophilized fibrinogen powder can also be used in powder form as a local hemostatic agent. If the lyophilized preparation is to be used in this fashion, it may be sprinkled directly onto a wound site or surgical incision where it reacts with endogenous thrombin to effect hemostasis. This is typically useful when the vessel or wound to be closed is small, and blood loss is not rapid.

Alternatively, the fibrinogen and thrombin may be applied to the wound or surgical incision by incorporation into a gauze pad, sponge, collagen or

gel-type matrix or into a similar device and treating the area to cause hemostasis or adhesion as necessary.

The fibrinogen based adhesive described herein affords a number of significant advantages over conventional surgical techniques (e.g., suturing) used alone as a surgical closure means and in combination with other techniques. The adhesive described herein provides a matrix for platelet adhesion and cell migration. When the preparation is applied topically to an actively bleeding site, the fibrin matrix effectively traps platelets, and through a series of enzymatic reactions, contributes to the biochemical cascade which culminates in clot formation.

Additionally, the fibrin matrix provides a compatible medium for the growth of contiguous cellular tissue. In this manner, cells from surrounding "like" tissue can infiltrate the matrix, thus providing healing and damaged cell replacement. The fibrin matrix thus supplements the repair integrity of identical tissue, resulting in the formation of a stronger bond with less scar tissue incorporated. The architecture of the effected tissue is therefore substantially restored to that of neighboring tissue by the cells migrating from the surrounding tissue sites.

Patients may be tested for skin test reactivity before the fibrinogen based adhesive is used to ensure that the individual will not demonstrate an untoward allergic reaction.

EXAMPLE

Plasma is collected in sterile one liter plastic bags containing 3.8% sodium citrate. PEG 30% solution is prepared by dissolving 300 grams of

polyethylen -8000 in water for irrigation (WFI) and bringing to one liter volume. Glycine is prepared by weighing 157.5 grams in a 1000 ml beaker. One volume is required for each liter volume of plasma processed. The following materials are weighed and dissolved in WFI:

1. Sodium chloride 49.49 gm
2. Sodium citrate 15.60 gm
3. Sodium phosphate 14.40 gm
4. EACA 78.60 gm

In a 6 liter Erlenmeyer flask the above ingredients are dissolved in 3 liters WFI. With 5 N HCl or NaOH, the pH is adjusted to 7.0. After pH adjustment, the volume is brought to 6.0 liters with WFI. The following procedure is carried out using asceptic technique:

Plasma (3.4 liters) is poured into a six liter beaker and a magnetic stir bar added. The magnetic stirrer is started and maintained at a moderate speed so as to not cause foam to develop in the flask. The first precipitation is started by gradually adding 600 ml of the 30% PEG solution. The PEG is added by six 100 ml volumes. Each 100 ml volume is added over a four minute period.

At the end of the precipitation the plasma and precipitate are poured into four sterile one liter polypropylene centrifuge bottles and capped. The samples are centrifuged at 4000 RPM at a temperature of about 2°C to 8°C for 20 minutes.

At the end of the centrifugation, the supernatant is decanted and the precipitate asceptically scraped from the bottles and added to a sterile six liter flask. The bottles are washed with buffer to recover as much of the precipitate as possible. The resulting slurry is brought to a 3.4 liter volume with buffer A. A magnetic stir bar is added and the stirrer is maintained at constant

speed until the precipitate is dissolved.

In the six liter flask containing the redissolved precipitate, a second precipitation is accomplished by adding 600 ml of 30% PEG at a rate of 100 ml over four minutes. At the end of the precipitation the buffer and precipitate are poured into sterile one liter polyproplylene centrifuge bottles and capped. The samples are centrifuged at 4000 RPM at a temperature of about 2°C to 8°C for 20 minutes.

At the end of the centrifugation, the supernatant is decanted and the precipitate asceptically scraped from the bottles and added to a sterile six liter flask. The bottles are washed with buffer A to recover as much of the precipitate as possible. The resulting slurry is brought to a 3.4 liter volume with Buffer A. A magnetic stir bar is added and the stirrer maintained at constant speed until the precipitate is dissolved.

The final precipitation is accomplished by gradually adding 157.5 grams of sterile glycine per liter, in a dry state, to the redissolved precipitate over a 20 minute period with constant stirring. The resulting precipitate and solution are again aseptically transferred to one liter centrifuge bottles and centrifuged at 4000 RPM at a constant temperature of about 2°C to 8°C for about 20 minutes.

The supernatant is decanted. The precipitate is transfered asceptically to a one liter screw cap Erlenmeyer flask. As much of the precipitate is removed as possible by washing with Buffer A. The transferred precipitate is dissolved with Buffer A and brought to a volume of 10% of the original volume of plasma, i.e. 10% of 3.4 liters of plasma is 340 ml.

With asceptic technique, the final volume of

product is filtered through a .22 micron filter. The filtered volume is aliquoted asceptically in a Laminar hood; 5 milliliters per vial. Each vial is stoppered and prepared for lypholization.

The plasma subfractions as described above can be further treated by established fractionation techniques to remove proteins that normally co-purifiy with fibrinogen and FXIII in the PEG/Glycine fractions (e.g. prothrombin, gamma globulins, albumin, fibronectin, plasmin, plasminogen, etc.).

While certain preferred embodiments of the invention are described herein in detail, numerous alternative embodiments are contemplated as being within the scope of the invention.

WHAT IS CLAIMED IS:

1. A process for producing a fibrinogen adhesive or hemostatic agent comprising:

precipitating the fibrinogen from plasma upon the addition of buffered polyethylene glycol;

reprecipitating the fibrinogen from solution upon the addition of buffered glycine, and

lyophilizing the fibrinogen to form a powder.

2. A process as defined in claim 1 wherein the plasma comprises bovine plasma.

3. A process as defined in claim 1 wherein the polyethylene glycol is polyethylene glycol-8000.

4. A process as defined in claim 1 or 3 wherein the polyethylene glycol is added in the form of a buffered solution containing citrate ions.

5. A process as defined in claim 4 wherein the buffer comprises monosodium phosphate, sodium chloride, sodium citrate and epsilon aminocaproic acid.

6. A fibrinogen-containing adhesive composition made by a process according to claim 1.

7. In a process of purifying fibrinogen, wherein the fibrinogen is precipitated from bovine plasma, an improvement which comprises adding to bovine plasma an amount of buffered PEG-8000 which is effective for precipitating the fibrinogen from the plasma.

8. The process of claim 7 wherein the fibrinogen precipitated with PEG-8000 is dissolved in a buffered solution and precipitated from solution with buffered glycine.

9. The process of claim 7 or 8 wherein the fibrinogen is lyophilized after precipitation.

10. A test kit for detecting a mammalian allergic reaction to bovine fibrinogen, comprised of

an injectable dose of a composition containing bovine fibrinogen and a diluent.

11. The test kit of claim 9 wherein the dose of bovine fibrinogen is present in an amount ranging from about 0.001 to about 100 mgs.

12. The test kit of claim 9 wherein the bovine fibrinogen is precipitated from bovine plasma with buffered PEG-8000.

13. A test kit as defined in claim 11 wherein the bovine fibrinogen is further purified by precipitation with buffered glycine.

14. A method of determining allergic reactivity to bovine fibrinogen comprising injecting a mammalian patient with an amount of bovine fibrinogen which is effective for inducing an allergic response, and

comparing the allergic response to a standard.

15. A method of inducing hemostasis in a mammalian patient with a site in need of such treatment, comprising applying to the site a hemostatic-effective amount of a fibrinogen composition which has been purified from bovine plasma by precipitation with buffered PEG-8000 and further purified by precipitation with buffered glycine.

16. The method of claim 15 further comprising applying an amount of thrombin to the wound site which is effective for converting the fibrinogen to fibrin.

17. The method of claim 16 wherein the fibrinogen composition and thrombin are combined and then applied to the wound site.

18. The method of claim 16 wherein the thrombin and fibrinogen are applied to the wound site separately.

19. The method of claim 17 wherein the

thrombin and fibrinogen are applied to the wound site with a gauze pad, sponge or gel matrix.

20. A method of closing a wound or surgical incision in a mammalian patient in need of such treatment, comprising applying to the wound or incision a composition containing fibrinogen which has been purified from bovine plasma by precipitation with buffered PEG-8000 and further purified by precipitation with buffered glycine.

21. A fibrinogen composition comprised of at least about 90 to about 98 percent fibrinogen, about 0.5 to about 2 to 3 percent fibrin, less than about 1 percent fibronectin and less than about 1 percent factor XIII.

22. A fibrinogen composition produced according to the process of claim 2, 3, 4 or 5.

23. In a process of producing a fibrinogen composition wherein the fibrinogen is precipitated from plasma with buffered polyethylene glycol, purified by precipitation with buffered glycine and redissolved, an improvement which comprises lyophilizing the redissolved precipitate at a cryogenic temperature while heating the dissolved precipitate to about the eutectic point for the precipitate.

24. The improved process described in claim 23 wherein the cryogenic temperature ranges from about -50°C to about -196°C.

25. The improved process of claims 23 or 24 wherein the composition is maintained at a cryogenic temperature for a period of about four hours to about 1 week.

26. The improved process of claim 25 further comprised of gradually increasing the temperature of the composition after lyophilization to about room temperature.

27. A fibrinogen composition in

accordance with claim 1, 21 or 23 in lyophilized form.

28. A process according to claim 1 wherein said plasma is pretreated to remove proteins that copurify with fibrinogen and FXIII in a PEG\glycine fraction.

29. A process according to claim 28 wherein said removed proteins are selected from the group consisting of prothrombin, gamma-globulin, fibronectin, plasmin, plasminogen and albumin.

30. A process according to claim 1 further comprising a step, subsequent to the step of precipitating the fibrinogen from plasma, of removing from the precipitate one or more proteins that copurify with fibrinogen and FXIII in a PEG/glycine fraction.

31. A process according to claim 30 wherein said protein is selected from the group consisting of prothrombin, gamma-globulin, fibronectin, plasmin, plasminogen and albumin.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US92/00931

I. CLASSIFICATION F SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): A61B 17/4; A61K 9/14
US CL: 514/2,8,21; 530/364,381,382

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
U.S.	514/2, 8, 21; 530/364, 381, 382

Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched[5]

APS

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
Y	US, A, 4,177,188 (Hansen) 04 December 1979. See columns 1-2, claims 1 and 2.	1-9, 22-3
Y	US, A, 4,298,598 (Schwarz et al.) 03 November 1981. See abstract.	1-31
X	US, A, 4,627,879 (Rose et al.) 09 December 1989. See columns 6 and 7, claims 12-19.	10-13

* Special categories of cited documents:[15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Report[2]
21 MAY 1992	16 JUN 1992
International Searching Authority[1]	**Signature of Authorized Officer**[20]
ISA/US	P. LYNN TOUZEAU

Form PCT/ISA/210 (second sheet)(May 1986) ß